

Give it or take it: the flux of one-carbon in cancer cells

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The sequence of the human genome together with sequence similarity analyses has advanced the discovery of missing steps in the mitochondrial one-carbon metabolism pathway. That together with the revived interest in cancer metabolism has brought the research on one-carbon metabolism back under the spotlight. Here, we present a brief review of recent advances in the field of one-carbon metabolism, with a bias towards its relevance to cell growth and proliferation in human cancers. We will address the requirements of one-carbon metabolism for biosynthesis and the major sources to satisfy that demand. We will also discuss some recent discoveries indicating a role of one-carbon metabolism beyond biosynthesis. We conclude with a concise enumeration of some fundamental questions that remain unanswered.

Introduction

The one-carbon metabolism pathway is composed of biochemical reactions that mediate the transfer of a one-carbon moiety (or unit) from donor to acceptor molecules, using folate as a cofactor [1]. In mammalian cells, serine, glycine, formate, choline, betaine, sarcosine and histidine are potential donors of one-carbon units and purines, methionine and thymidylate are potential acceptors. Mammalian cells can also release the one-carbon unit to extracellular media as formate or CO₂. Folate cofactors are found in

different forms, from the pure or primary folate (pteroyl monoglutamate, vitamin B₉) to derivatives with different levels of oxidation/reduction and different number of glutamate groups (polyglutamates). The reduction of folates facilitates its transport across membranes and the addition of glutamate groups enhances its retention in cellular compartments.

One-carbon metabolism is essential to satisfy the biosynthetic demands of highly proliferative mammalian cells. Examples are the rapid cell population

Abbreviations

10-formyl-THF, 10-formyl-tetrahydrofolate; 2HG, (D)2-hydroxyglutarate; 5,10-CH₂-THF, 5,10-methylene-tetrahydrofolate; AML, acute myeloid leukaemia; AMPK, AMP kinase; AMT, aminomethyltransferase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; DHFR, dihydrofolate reductase 1L; DLD, dihydrolipoyl dehydrogenase; dTMP, thymidylate; FTHFD, 10-CHO-THF dehydrogenase; FTHFS, 10-formyl-tetrahydrofolate synthetase; GCS, glycine cleavage system; GCSH, glycine cleavage system protein H; GLDC, glycine decarboxylase; IDH1, isocitrate dehydrogenase 1; IDH2, isocitrate dehydrogenase 2; MAT, methionine adenosyltransferase; MTHFD1, 5,10-methylene-tetrahydrofolate dehydrogenase/5,10-methylene-tetrahydrofolate cyclohydrolase 1; MTHFD1L, 5,10-methylene-tetrahydrofolate dehydrogenase/5,10-methylene-tetrahydrofolate cyclohydrolase 1L; MTHFD2, 5,10-methylene-tetrahydrofolate dehydrogenase/5,10-methylene-tetrahydrofolate cyclohydrolase 2; MTHFD2L, 5,10-methylene-tetrahydrofolate dehydrogenase/5,10-methylene-tetrahydrofolate cyclohydrolase 2L; MTHFR, 5-methyl-tetrahydrofolate reductase; NSCLC, non-small cell lung cancer; PHGDH, 3-phosphoglycerate dehydrogenase; PSAT1, phosphoserine aminotransferase 1; ROS, reactive oxygen species; SAM, S-adenosylmethionine; SHMT1, serine hydroxymethyltransferase 1; SHMT2, serine hydroxymethyltransferase 2; THF, tetrahydrofolate; TYMS, thymidylate synthetase.

expansion during embryonic development [2], T-cell lymphocyte activation [3,4] and during rapid tumour growth [5]. These rapidly proliferating cells require one-carbon metabolism for the *de novo* synthesis of purines and thymidylate. In contrast, as discussed here, methionine synthesis is negligible in proliferating cancer cells, where methionine is instead imported from the serum at rates sufficient to satisfy the biosynthetic demand.

In mammalian cells, and eukaryotes in general, one-carbon metabolism is partitioned between reactions taking place in the cytosol or in the mitochondria [1]. Although there is some level of isomorphism between the cytosolic and mitochondrial pathways, there are some differences. In this review, we summarize recent discoveries regarding the compartmentalization of mammalian one-carbon metabolism in the context of cancer. The discussion will be focused around the flow of one-carbon units rather than on folate metabolism. For a more comprehensive review on the subject, touching on the evolution of one-carbon metabolism, we recommend the excellent review by Tibbetts and Appling [1]. We will discuss the state of the art of one-carbon metabolism in the cancer field.

Compartmentalization of one-carbon metabolism

Mammalian cells have complementary pathways for one-carbon metabolism in the cytosol and the mitochondria (Fig. 1). Serine and glycine are the major donors of one-carbon units. Serine hydroxymethyltransferase (SHMT) transfers a one-carbon moiety from serine to tetrahydrofolate (THF), forming 5,10-methylene-THF (5,10-CH₂-THF) and glycine. The gene *SHMT1* encodes the cytosolic SHMT (SHMT1) [6], whereas *SHMT2* encodes two isoforms, mitochondrial SHMT (SHMT2) and cytosolic/nuclear SHMT (SHMT2 α) [7]. In the mitochondria, the glycine cleavage system transfers a one-carbon moiety from glycine to 5,10-CH₂-THF, releasing CO₂ and ammonia. The glycine cleavage system is a complex of the T-, L-, P- and H-protein. Protein P has glycine decarboxylase activity and it is encoded by *GLDC*. Protein T has aminomethyltransferase activity and it is encoded by *AMT*. Protein L has dihydrolipoyl dehydrogenase activity and it is coded by *DLD*. Finally, protein H, encoded by *GCSH*, shuttles the methylamine group of glycine from the P to the T protein.

5,10-CH₂-THF is converted back to THF through different folate cycles, depending on the final one-carbon acceptor. On one folate cycle, the bifunctional 5,10-methylene-tetrahydrofolate dehydrogenase/5,10-

methylene-tetrahydrofolate cyclohydrolase (MTHFD) enzyme converts 5,10-CH₂-THF to 10-formyl-THF (10-CHO-THF) (Fig. 1 FOR cycle). There are two homologous genes encoding bifunctional MTHFDs that localize to the mitochondria, *MTHFD2* and *MTHFD2L* encoding MTHFD2 and MTHFD2L respectively. MTHFD2 is expressed in highly proliferative cells, during embryonic development and in cancer cells [8–11]. *In vitro* biochemical studies indicate that MTHFD2 activity is predominantly NAD⁺-dependent [12]. MTHFD2L is expressed in adult tissues and *in vitro* biochemical studies indicate that it has similar NAD⁺/NADP⁺-dependent activities [13,14]. 10-CHO-THF is converted back to THF via either reverse 10-CHO-THF synthase (FTHFS), releasing the one-carbon as formate, or via 10-CHO-THF dehydrogenase (FTHFD), releasing the one-carbon as CO₂. There is a mitochondrial FTHFS encoded by *MTHFD1L* [15] and cytosolic/mitochondrial FTHFDs encoded by *ALDH1L1/ALDH1L2* respectively [16,17]. In the cytosol, a single gene product from the *MTHFD1* gene encodes a trifunctional enzyme with MTHFD and FTHFS activities [18]. A function of MTHFD1 is to recapture the formate released by mitochondrial MTHFD1L to form cytosolic 10-CHO-THF, which is then used in two different steps of purine synthesis, recovering back THF [1].

On another folate cycle, thymidylate synthase (TS) transfers a one-carbon from 5,10-CH₂-THF to dUMP, forming thymidylate (dTMP) and dihydrofolate (DHF) (Fig. 1, dTMP cycle). DHF reductase (DHFR) converts DHF back to THF. Thymidylate synthesis takes place in both the mitochondria and the nucleus (Fig. 1), supporting the synthesis of mitochondrial and cytosolic DNA respectively. A single gene, *TYMS*, encodes a gene product that localizes to both the cytosol and the nucleus and it is responsible for the TS activity in these compartments. The product of the gene *DHFR* is responsible for the cytosolic/nuclear DHFR activity [19]. There is a second gene, *DHFRLL1*, that encodes a protein that localizes to the mitochondria and it is responsible for the DHFR activity in this compartment [20]. Finally, purine and methionine synthesis are localized in the cytosol.

One-carbon metabolism is essential for development

The metabolism of cancer resembles the metabolism of growth and development in many aspects [21]. With this perspective in mind, we can learn about the essentiality of one-carbon metabolism enzymes by studying their requirements during embryonic development.

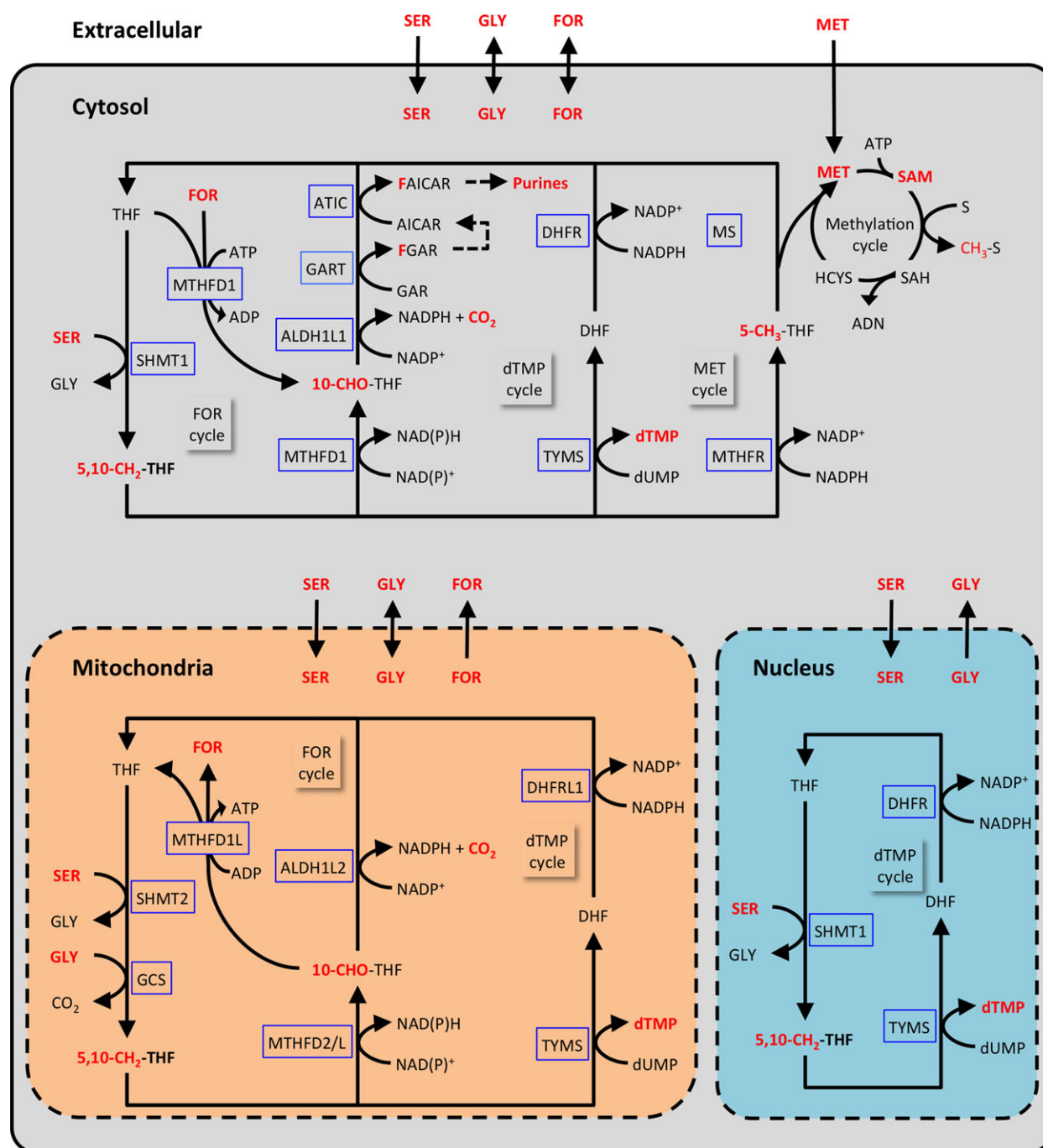


Fig. 1. Compartmentalization of one-carbon metabolism in mammalian cells. Different compartments are highlighted with different background colours. Metabolic enzymes are enclosed in boxes. Arrows indicated the flow of one-carbon units based on current evidence.

Homozygous *Mthfd2* knockout mice die *in utero*. Homozygous deletion of either *Mthfd2* [22], *Mthfd11* [2], or *Amt* [23] causes embryonic lethality and neural tube defects in mice. However, knock-down experiments in cancer cells *in vitro* have resulted in conflicting outcomes. *MTHFD1L* or *MTHFD2* knock-down

inhibits growth and viability of the nonmetastatic breast cancer cell line MCF7 [24]. In contrast, *MTHFD2* knock-down does not impair the growth and viability of the highly metastatic breast cancer lines MDA-MB-231 and BT-549 [24,25], although it affects their invasive potential. It is possible that

different cancer types have different degrees of dependency on these mitochondrial one-carbon metabolism enzymes for proliferation. For example, we cannot exclude that in some cancer cells, the cytosolic pathway is able to compensate for the inhibition of the mitochondrial pathway. Finally, it cannot be discounted that the embryonic lethality and neural tube defects observed in mice deficient in mitochondrial one-carbon metabolism genes are not linked to cell growth but to tissue remodelling during development.

Serine is the main donor of one-carbon units

Early investigations interrogating the nutrient requirements of activated human lymphocytes demonstrated that serine is the main donor of one-carbon units in proliferating normal cells [3,4]. Serine deprivation arrests activated lymphocytes in the G1 phase of the cell cycle, and growth resumes upon addition of serine [3]. Other potential sources of one-carbon units such as glycine, choline and histidine do not rescue growth, and formate only partially rescues growth upon serine starvation [4].

The discovery of signalling pathways regulating this cell cycle arrest had to wait until the advent of molecular biology research and the renewed interest in cancer metabolism. In cancer cells, cell cycle arrest due to serine starvation is p53-dependent [26]. Cancer cells lacking p53 do not arrest upon serine starvation. Instead they die due to a mismatch between the supply and demand of glycine [26]. As observed in activated lymphocytes, glycine does not rescue serine starvation of cancer cells. In contrast, addition of exogenous glycine inhibits growth [27]. Mechanistically, in the absence of extracellular serine, the activity of SHMT is reverted from glycine producing to glycine consuming, from production to consumption of one-carbon units. As a consequence, there is a depletion of the one-carbon unit pool and inhibition of purine synthesis. Addition of formate rescues growth, demonstrating the inhibition of purine synthesis as the mechanism of growth inhibition [27]. There was an earlier report suggesting that glycine supports rapid proliferation in cancer cells [28]. However, those observations are most likely an artefact of serine depletion during the course of the experiments, which affects mostly the results for rapidly proliferating cells.

In mammalian cells, serine can be taken up from the extracellular medium or it can be synthesized from glucose. *In vitro* studies indicate that proliferating cells use both sources but with a higher flux of serine uptake relative to serine synthesis from glucose [26,29].

In cancer cells, serine is actually imported at a rate that exceeds the serine demand and the excess serine is converted to glycine [30]. However, some cancer cells have amplifications of the *PHGDH* gene [31,32], encoding the enzyme 3-phosphoglycerate dehydrogenase, the first step in the branching point from glycolysis to serine synthesis. Examples include the breast cancer cell lines MDA-MB-468, BT-20 and HCC70 [31] and the melanoma cell lines WM266-3, Malme and SK28 [32]. Cancer cells with *PHGDH* amplifications have higher levels of the gene product and a higher rate of serine synthesis from glucose. Down-regulation of *PHGDH* expression inhibits the growth of cancer cells with *PHGDH* amplifications but not of cells with normal *PHGDH* copy number. Why cancer cells with high *PHGDH* expression are dependent on the *PHGDH* metabolic activity remains an open question. Cancer cells could also import serine from the extracellular media [28,33], suggesting that the synthesis of the pathway end product, serine, is not the selected phenotype. The serine synthesis pathway contains a NAD^+ dehydrogenase step (catalysed by *PHGDH*), suggesting that this pathway could contribute NADH generation. However, as the rate of serine synthesis is low relative to the rate of glycolysis, even in cells with high *PHGDH* expression, glycolysis is the major source of NADH generation in the cytosol. The serine synthesis pathway also contains a transaminase step catalysed by phosphoserine aminotransferase (PSAT, encoded by *PSAT1* in humans), converting glutamate to α -ketoglutarate. In cancer cells with high *PHGDH* expression, the generation of α -ketoglutarate from the serine synthesis pathway is significant when compared with other pathways [31]. These data suggest that the selective advantage of high *PHGDH* expression is more likely linked to the contribution of serine synthesis to the glutamate/ α -ketoglutarate balance.

Relative contribution of cytosolic and mitochondrial pathways

Isotope tracing is the main tool to determine the direction of metabolic fluxes and to quantify the contribution of different pathways to the production of the same product. In ground-breaking work, Appling's laboratory investigated the incorporation of medium [$3\text{-}^{13}\text{C}_1$]-serine into yeast intracellular metabolites using NMR spectroscopy [34]. They observed significant amounts of intracellular [$2\text{-}^{13}\text{C}_1$]-glycine that could only be obtained by the reverse activity of mitochondrial glycine cleavage together with [$1\text{-}^{13}\text{C}_1$]-5,10- CH_2 -THF derived from mitochondrial SHMT activity.

Later, studies switched to $[2,3,3\text{-}^2\text{H}_3]$ -serine to tease out the relative contribution of the cytosolic and mitochondrial pathways. In a nutshell, the generation of cytosolic 5,10- CH_2 -THF can be done using the cytosolic or mitochondrial pathways. The cytosolic route is basically one step catalysed by SHMT1 and it produces $[^2\text{H}_2]$ -5,10- CH_2 -THF ($M + 2$) (Fig. 2, black arrows). In contrast, the mitochondrial route requires a dehydrogenase step where one deuterium (^2H) is released, resulting in $[^2\text{H}_1]$ -5,10- CH_2 -THF ($M + 1$) (Fig. 2, green arrows). The relative abundance of $M + 1/M + 2$ 5,10- CH_2 -THF is then reflected on the relative abundance of $M + 1/M + 2$ thymidylate and of $M + 1/M + 2$ methionine, which can both be quantified using mass spectrometry. In mouse embryonic fibroblasts, about 85% of deuterated methionine is $M + 1$ and it goes down to about 23% in *Mthfd2* null cells [15]. In breast cancer MCF7 cells, about 97.8% of deuterated methionine and about 93.3% thymidylate are $M + 1$ [35]. These data strongly support the mitochondrial pathways as the main route of formation of cytosolic one-carbon units.

More recently, a reported assay has been developed exploiting the discovery of neomorphic mutations in *IDH1* and *IDH2* [36]. The cancer genome sequencing projects uncovered *IDH1* and *IDH2* mutations in the context of human cancers, including acute myeloid

leukaemias (AML) [37] and gliomas [38,39]. These mutations were found at specific amino acid residues, R132 in *IDH1* or R140 and R172 in *IDH2*, suggesting some functional relevance. Indeed, follow-up biochemical studies demonstrated that these mutations result in neomorphic *IDH* enzymes catalysing the conversion of α -ketoglutarate to (D)2-hydroxyglutarate (2HG) with the concomitant transfer of a hydrogen atom from NADPH to 2HG [40,41]. In *IDH1/2* wild-type cells, 2HG is found at very low levels. In contrast, expression of the neomorphic *IDH1/2* mutant enzymes results in high 2HG concentrations. As *IDH1* localizes to the cytosol and *IDH2* to the mitochondria, cells transfected with *IDH1* or *IDH2* mutant enzymes can be utilized to trace ^2H from a ^2H -labelled nutrient to NADPH in the cytosolic and mitochondrial compartments respectively (Fig. 2, blue boxes). Specifically, in cells transfected with *IDH1* mutant enzyme, the 2HG $M + 1$ fraction serves as a readout of the SHMT/MTHFD activity in the cytosol, and in cells transfected with *IDH2* mutant enzyme, the 2HG $M + 1$ fraction serves as a readout of the SHMT/MTHFD activity in the mitochondria. Using this reported assay, it has been confirmed that in cancer cells, serine is the major one-carbon donor and the SHMT/MTHFD activity is mostly mitochondrial [36].

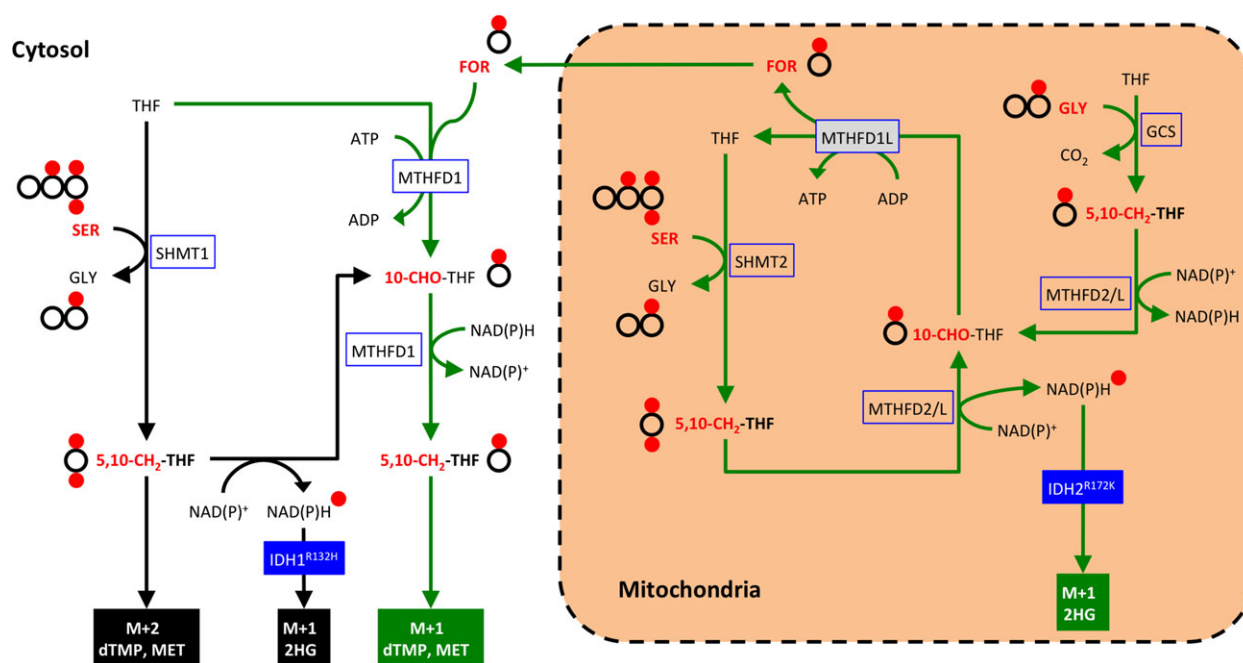


Fig. 2. $[2,3,3\text{-}^2\text{H}_3]$ -serine tracing to determine the relative contribution of cytosolic (black lines) and mitochondrial (green lines) one-carbon metabolism pathways. The black empty circles represent carbon atoms and the red-filled circles represent ^2H atoms. Metabolic enzymes are enclosed in boxes and are highlighted with a blue background for mutant *IDH1/2* enzymes.

The predominant use of the mitochondrial SHMT/ MTHFD pathway in proliferating cancer cells is further corroborated by protein expression profiles. Using proteomic data reported for the NCI panel of 60 cancer cell lines [42], we have quantified the relative abundance of the different proteins in the cytosolic and mitochondrial one-carbon metabolism pathways (Fig. 3). SHMT2 is by far the most abundant protein in all cell lines. At an intermediate relative level of expression, we find MTHFD2, ALDH1L2, GLDC and GCSH, all localizing to the mitochondria. In contrast, the expression of the cytosolic SHMT1 and MTHFD1 proteins is relatively small. The protein expression analysis also reveals that some cell lines do express relatively high levels of the glycine cleavage components GLDC and GCSH. Future research should determine whether glycine substitutes for serine as a one-carbon donor in these cancer cell lines.

The pattern of expression is different in somatic tissues and it depends on the tissue type [14]. In the kidney and liver of adult mice, *Mthfd1* gene expression is the highest, with negligible expression of the mitochondrial pathway genes *Mthfd2*, *Mthfd2l* and *Mthfd1l*. In contrast, in the brain and the spleen, *Mthfd1l* exhibits levels of expression similar or higher to *Mthfd1*. While it is not straightforward to extrapolate from the expression of metabolic enzyme genes to actual metabolic fluxes, these data indicate that the cytosolic and mitochondrial one-carbon metabolism pathways are used differently in nonproliferating somatic tissues and that there are differences across tissue types.

Roles of glycine cleavage

As we have just discussed, most of the reported data indicate serine as the main donor of one-carbon units

in cancer cells. Experiments culturing baby mouse kidney cells (iBMK) [43] or HCT116 lung cancer cells in culture medium with ^{13}C -labelled glycine have shown that glycine cleavage does not contribute to the generation of one-carbon units in these cells. Yet, there is also evidence pointing to a role for the glycine cleavage system (GCS) in cancer, albeit not as a major one-carbon donor. Overexpression of GLDC, the enzyme catalysing the first glycine decarboxylase step of the GCS, drives tumour formation in lung adenocarcinoma [44]. Knock-down of *GLDC* in lung adenocarcinoma-derived A549 cells results in significant changes in the levels of several metabolites and inhibits proliferation. Among the altered metabolites, sarcosine levels significantly decreased upon *GLDC* knock-down in A549 cells. In turn, supplementation with 10 μM sarcosine was sufficient to rescue the growth inhibition caused by *GLDC* knock-down. It remains unclear how *GLDC* knock-down results in reduced sarcosine levels. In human cells, glycine and sarcosine can be interconverted by sarcosine dehydrogenase and glycine N-methyltransferase. Knock-down of *GLDC* results in increased levels of glycine [44], in principle favouring the formation of sarcosine. Instead, a decrease of sarcosine levels was observed [44]. However, given that the *GLDC* knock-down leads to several metabolic changes, there could be other rearrangements favouring a decrease in sarcosine levels.

In glioblastoma cells with high SHMT2 activity, glycine cleavage protects cells from toxicity associated with high glycine levels [45]. Knock-down of *GLDC*, the first glycine decarboxylase step of the GCS, results in loss of viability and breakdown of neurospheres formed by glioblastoma-derived cell lines. The loss of viability was tracked down to the toxic effects

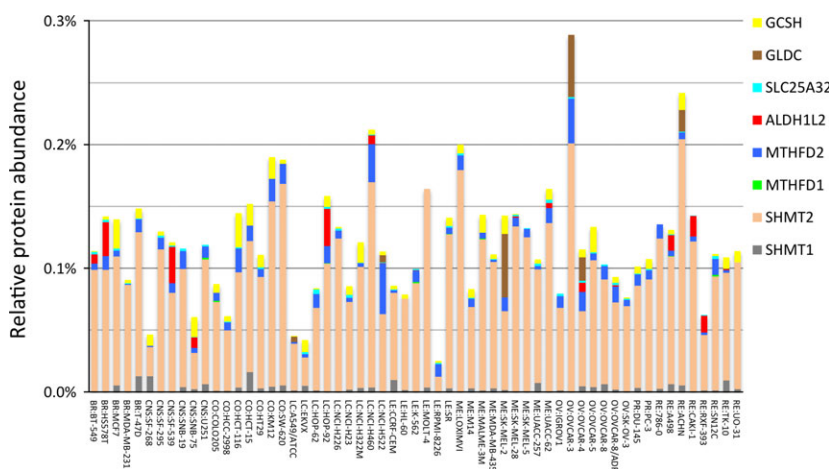


Fig. 3. Expression of one-carbon metabolism proteins across a panel of tumour-derived cell lines, based on proteomic data previously reported [42]. The bars represent the sum of intensities of peptides mapping to the corresponding protein relative to the sum of intensities of all detected peptides. The levels of MTHFD1L and ALDH1L1 were below detection limit in all samples.

of the glycine metabolism by-products. Mechanistically, the presence of an active GCS prevents the accumulation of high glycine levels. Inhibition of the GCS leads to increased glycine levels favouring the occurrence of less efficient reactions that do not take place at a significant rate at low glycine levels. Knock-down of *GLDC* or glycine overload results in an increase of aminoacetone and methylglyoxal levels in a glycine C-acetyltransferase (GCAT)-dependent manner [45]. Taken together this evidence points to a protective role of the GCS in cells with high SHMT2 activity.

Interactions with methylation metabolism

One-carbon metabolism intersects with methylation metabolism (Fig. 1, MET and methylation cycles). The activity of 5-methyl-THF (5-CH₃-THF) reductase (MTHFR) converts 5,10-CH₂-THF to 5-CH₃-THF. The methionine synthase (MS) then transfers a one-carbon unit from 5-CH₃-THF to homocysteine forming methionine, the source of methyl groups in mammalian cells (Fig. 1, methylation cycle). In mouse embryonic fibroblasts cultured in medium containing [2,3,3-²H₃]-serine only, about 4% of methionine is deuterated [15], indicating extracellular methionine as the main source of intracellular methionine. Tracing experiments with ¹³C-labelled serine or methionine have shown that extracellular methionine is the main source of methionine in proliferating cancer cells as well [29,33,46,47]. Taken together this evidence indicates that in proliferating mammalian cells and in cancer cells, one-carbon metabolism has a negligible contribution to the generation of methionine.

However, to donate the methyl group, methionine needs to be first converted to S-adenosylmethionine (SAM), the universal methyl donor in methylation reactions. SAM is synthesized by the methionine adenosyltransferase (MAT)-dependent ligation of a methionine molecule and an ATP molecule. The requirement of an ATP molecule as chemical group (not an energy currency) for the synthesis of SAM prompted Maddocks *et al.* [33] to investigate methylation metabolism in the context of serine starvation. As discussed above, in cancer cells, serine is the major source of one-carbon units for purine synthesis, and serine deprivation results in a significant drop in purine levels. Maddocks *et al.* were able to show that the inhibition of purine synthesis by serine deprivation significantly impairs RNA/DNA methylation by reducing the availability of ATP for the synthesis of SAM.

Nonbiosynthetic roles

The evidence reviewed so far demonstrates the requirement of one-carbon metabolism for *de novo* synthesis of purines and thymidylate. However, recent data suggest that one-carbon metabolism has roles beyond biosynthesis. First, in proliferating cancer cells, the generation of one-carbon units from serine exceeds the one-carbon demand for purine synthesis [29]. Second, there are reactions in one-carbon metabolism that can generate ATP and NADPH, potentially contributing to the energy and antioxidant demands of proliferating cells.

The investigation of *in silico* models of human cell metabolism predicts that serine catabolism can generate ATP via the activity of reverse FTHFS (MTHFD1 or MTHFD1L) [48] (see Fig. 1). There is currently no direct experimental evidence for a net production of ATP by the overall activity of cytosolic and mitochondrial reverse FTHFS activity. Yet, treatment of cancer cells with the antifolate methotrexate results in energy stress, characterized by an increase of the AMP/ATP ratio and activation of AMP kinase (AMPK) [9], providing some indirect evidence.

The investigation of *in silico* models of human cell metabolism also predicts that serine catabolism can generate NADPH via the activity of cytosolic and mitochondrial MTHFD and FTHFD (see Fig. 2) [29,43,48]. This prediction is corroborated by the experimental quantification of NADPH production fluxes in cancer cells, albeit with some differences in the differential utilization of the cytosolic and mitochondrial pathways. In immortalized baby mouse kidney cells (iBMK) cultured in [2,3,3-²H₃]-serine, there is a significant labelling of NADPH from media [2,3,3-²H₃]-serine [43]. Furthermore, *Mthfd1* knock-down drastically reduced the NADPH labelling from serine, suggesting that NADPH production was from the cytosolic pathway.

Studies using the IDH1/IDH2 reporter assays provide additional evidence for NADPH production from serine catabolism. As IDH1 and IDH2 are predominantly NADP⁺-dependent dehydrogenases, the fraction of ²H-2HG in cells cultured with [2,3,3-²H₃]-serine quantifies the relative contribution of serine one-carbon catabolism to the generation of NADPH (Fig. 2, blue boxes). Furthermore, using cells transfected either with mutant IDH1 or IDH2, it is possible to tease out the relative contribution to the cytosolic and mitochondrial NADPH pools. In experiments done with H1299 and A549 lung cancer cell lines, there was a significant labelling of 2HG from [2,3,3-²H₃]-serine only in cells transfected with an IDH2-encoding plasmid, indicating

that in these cells serine catabolism contributes to NADPH generation in the mitochondria.

The observed differences between NADPH production in the cytosol of kidney cells versus NADPH production in the mitochondria of proliferating cancer cells are consistent with the protein expression differences highlighted above. In cancer cells, there is a predominant expression of the mitochondrial pathway proteins (particularly SHMT2, Fig. 3), while in the kidney and liver of mice, MTHFD1 is highly expressed compared to all other tissues and all other one-carbon metabolism proteins.

The potential contribution of one-carbon metabolism to NADPH generation is consistent with the observation that NRF2, a master transcription factor of antioxidant response, controls the expression of genes in one-carbon metabolism in nonsmall cell lung cancer (NSCLC) cells with high NRF2 expression (e.g., A549) [49]. In mammalian cells, glutathione (GSH) oxidation/reduction coupled to NADPH reduction/oxidation is a major pathway for ROS detoxification. One-carbon metabolism can contribute ROS detoxification in two ways. The transcription factor NRF2 regulates the expression of several genes involved in ROS detoxification. The fact that it also regulates the expression of genes in the serine synthesis pathway (*PHGDH*, *PSAT1*) and one-carbon metabolism (*SHMT2*) supports these roles. It is worth noticing that this regulation is indirect, via the activity of ATF4, a master regulator of cell response to amino acid starvation.

Outlook

The discovery of missing steps in the mitochondrial one-carbon metabolism pathway together with the revived interest on cancer metabolism has brought one-carbon metabolism back under the spotlight. Recent studies have significantly advanced our understanding of the differential utilization of cytosolic and mitochondrial pathways by cancer cells. In turn, this body of work has left open some fundamental questions.

There is evidence indicating that one-carbon metabolism contributes to NADPH generation [36,43]. Yet, it is not clear how much of that contribution is a by-product of unavoidable dehydrogenase steps in biosynthetic pathways combined with a limited NADH oxidation capacity or a *bona fide* NADPH generation to fulfil both the biosynthetic and antioxidant NADPH demand.

Theoretical models suggest that the reverse activity of cytosolic or mitochondrial FTHFS can produce ATP with concomitant one-carbon release as formate [48]. If this theoretical prediction was proven true, it

would imply serine one-carbon catabolism as a third pathway for energy generation in mammalian cells, besides the established glycolysis and mitochondrial oxidative phosphorylation.

We have a limited understanding about the role of glycine cleavage in cancer. There is some evidence supporting a tumour-promoting role in the formation of lung adenocarcinoma [44] and a protective role in glioblastomas with high SHMT2 expression [45]. However, it is not clear whether these two observations are connected and what their relevance is in other cancer types. Overall, we currently have a poor understanding of mitochondrial glycine metabolism in cancer.

Finally, it is established that mitochondrial one-carbon metabolism is the source of cytosolic one-carbon units in proliferating yeast [34,50], mammalian [1,3,4] and cancer cells [36]. Yet, we do not understand what is the evolutionary advantage of this choice. Purine synthesis is the major demand of one-carbon units in proliferating cells and all the purine synthesis enzymes are localized in the cytosol. The key question is why one-carbon metabolism uses the mitochondrial instead of the cytosolic one-carbon metabolism pathway. It cannot be excluded that the answer to some of the previous questions will shed light on the evolutionary advantage of a mitochondrial route for the generation of one-carbon units.

Author contributions

JM and AV reviewed the literature and wrote the manuscript.

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